In Vitro Cannabis Exposure Alters Human Granulosa Cell Extracellular Vesicle Release

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Extracellular vesicles (EVs) are very important for cell communication in the ovarian follicles and exogenous molecules could perturb these exchanges affecting crucial events involved in oocyte maturation and fertility. Indeed, cannabidiol (CBD), sometimes found in cannabis products, has been reported to decrease exosome release in cancer cells. Also, we have recently shown that cannabis metabolites (including tetrahydrocannabinol (THC)) can be found in the follicular fluid of patients undergoing IVF treatment. Thus, the objectives of this study were to determine if CBD and THC affect EVs release by granulosa cells.

Granulosa cells were obtained from consenting patients undergoing in vitro fertilization treatments with ethic approval (Veritas IRB #16518). $0.5x10^6$cells/mL were plated in a 6-well plate for 24h and then treated with EV inhibitors (Imidazole (10µM), GW4869 (5µM)), cannabis compounds (CBD 1µM) or a combination of THC metabolites, Δ9-THC 25ng/mL, 11-OH-THC 5ng/mL and 11-COOH-THC 50ng/mL). The next day, exosome-like particles were enriched using Total Exosome Isolation Reagent from conditioned cell culture media (Invitrogen), quantified using NanoSight NS300 and DynaPro Plate Reader III, protein concentrations were assessed using Qubit and exosome-like particle membrane protein content was assessed using MACSPlex Exosome Kit (Miltenyi).

There was no difference in the number of cells or cell viability was observed after 24h, but a significant decrease in the number particles size 25-150nm when cells were treated with Imidazole ($2.6x10^7$/mL, $p<0.01$), GW4869 ($1.7x10^7$/mL, $p<0.001$) and CBD ($4.2x10^7$/mL, $p<0.01$) compared to Control ($11.3x10^7$/mL). On the other hand, we observed a significant increase the number of particles when cells were treated with THC ($18.5x10^7$/mL, $p<0.01$) compared to Control (11.3x10^6/mL). When exosome-like particles were enriched, there was no differences in total protein content or exosome marker expression (CD9, CD63, and CD81). We observed a significant decrease in the expression of CD9, CD44, CD142, CD24 and CD41b of EVs enriched from the GW4869 treatment ($p<0.05$), but not for the other treatments (n=4).

CBD seemed to decrease the amount of EVs secreted while THC seems to increase it, but there was no change in their membrane protein content. Future investigations into the acute and chronic exposure have on EV secretion as well as the differences in protein and miRNA cargo are ongoing.